Mechanisms Underlying HIV Associated Non-infectious Lung Disease

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Abstract

Pulmonary disease remains a primary source of morbidity and mortality in persons living with HIV (PLWH), although the advent of potent combination antiretroviral therapy (cART) has resulted in a shift from predominantly infectious to noninfectious pulmonary complications. PLWH are at high risk for chronic obstructive pulmonary disease, pulmonary hypertension, and lung cancer even in the era of cART. The underlying mechanisms of this are incompletely understood, but recent research in both human and animal models suggest that oxidative stress, expression of matrix metalloproteinases, and genetic instability may result in lung damage which predisposes PLWH to these conditions. Some of the factors which drive these processes include tobacco and other substance use, direct HIV infection and expression of specific HIV proteins, inflammation, and shifts in the microbiome towards pathogenic and opportunistic organisms. Further studies are needed to understand the relative importance of these factors to the development of lung disease in PLWH.

Key words: HIV, inflammation, COPD, pulmonary hypertension, lung cancer

Abbreviations:

HIV: human immunodeficiency virus

AIDS: acquired immunodeficiency syndrome

cART: combination antiretroviral therapy

COPD: chronic obstructive pulmonary disease

HIV-PAH: HIV associated pulmonary arterial hypertension

IVDU: intravenous drug use

PFT: pulmonary function test

DLCO: diffusing capacity of the lung for carbon monoxide

BAL: bronchoalveolar lavage

PD-1: programmed death 1

EC: endothelial cells

MACS: multicenter AIDS cohort

SIV: simian immunodeficiency virus

AM: alveolar macrophages

INHALD: Investigating HIV associated mechanisms of lung disease consortium

PBMC: peripheral blood mononuclear cells

INTRODUCTION

Pulmonary complications remain a significant source of morbidity and mortality in patients infected with human immunodeficiency virus (HIV). Prior to the advent of combination antiretroviral therapy (cART), pulmonary infections, especially *Pneumocystis jiroveci*, tuberculosis and community acquired pneumonia, were among the leading causes of death [1]. With the advent of cART, non-infectious pulmonary complications have become more prevalent in persons living with HIV (PLWH), including chronic obstructive pulmonary disease (COPD), HIV related pulmonary artery hypertension (HIV-PAH) and lung cancer [2;3;4]. The prevalence of COPD in PLWH in the modern cART era varies by study and by definition, but by spirometry 7-9% of PLWH have clinical obstruction, while one third have respiratory symptoms [5;6]. The prevalence of PAH is 2500 times higher than in the general population, with 0.5% of PLWH estimated to have HIV-PAH, despite effective cART [7]. The incidence of lung cancer is 2.7 times higher in PLWH than in the general population [8;9]. The mechanisms underlying the increased rates of noninfectious lung disease are multifactorial. High rates of tobacco use and intravenous drug use (IVDU) likely contribute, in addition to chronic lung inflammation and subsequent oxidative stress and tissue damage. In this review, we discuss what is currently known about the pathogenesis of COPD, PAH, and lung cancer in PLWH, describe other potential relevant mechanisms of lung damage which may contribute to these diseases (Figure 1), and outline priorities for better understanding the underlying mechanisms in the current cART era.

HIV AND COPD

COPD is highly prevalent among cigarette smokers, and incidence increases with age [10]. As PLWH are living longer, high smoking rates contribute to increasing incidence of COPD [3]. Rates of hospitalization for obstructive lung disease have increased for PLWH in the cART era, and are expected to increase further as the population ages [2]. Several studies have demonstrated that PLWH have higher rates of dyspnea and alterations in pulmonary functions tests (PFT), particularly low diffusing capacity of the lung for carbon monoxide (DLCO), as well as imaging findings of emphysema[4;5;6]. PLWH also appear to have more respiratory symptoms, including dyspnea and cough, than smokers with similar disease burden by PFT criteria [6].

Inflammation plays an important role in the pathogenesis of COPD in PLWH. Reduced frequencies and absolute numbers of CD4+ T cells are seen in bronchoalveolar lavage (BAL) of PLWH with COPD [12]. These CD4+ T cells, but not CD8+ T cells, demonstrate impaired lung mucosal immunity to HIV, and express high levels of programmed cell death 1 (PD-1), a marker of immune activation and exhaustion. They also express high levels of the Fas death receptor, CD95, and demonstrate increased Fas-dependent activation induced cell death [12]. All of these findings result in progressive loss of CD4+ T cells in the BAL, leading to a profound imbalance in CD4:CD8 ratio and persistent CD8+ T cell alveolitis in HIV-associated COPD. This inflammation leads to the expression of inflammatory cytokines and release of matrix metalloproteinases associated with COPD [13;14].

HIV-PAH

The association between HIV infection and pulmonary hypertension was recognized early on in the AIDS epidemic [15]. Although cART may have decreased the incidence of HIV-PAH

and has been shown to partially reverse pulmonary hypertension in a small number of PLWH [7], PAH remains a significant clinical complication in PLWH, particularly as life expectancy increases. Symptoms and signs usually present late, and half of patients with HIV-PAH die during a median follow-up period of 8 months [16], although a more recent study demonstrated that three year survival is 72% in patients treated with both cART and specific PAH therapy [17]. Specific therapy for PAH appears to improve hemodynamic parameters more than cART, as cART has minimal effect even if it is instituted early in disease [18]. Approximately two-thirds of deaths are attributed to pulmonary hypertension rather than complications of immune deficiency [16;15]. CD4+ T cell count is the only independent predictor of survival, and patients with HIV-PAH have poorer survival rates when compared to uninfected patients with PAH [15;16].

Direct HIV infection, substance abuse and chronic inflammation are particularly important in the development of HIV-PAH [19]. Chronic exposure to HIV viral proteins in the lung (e.g. Nef, Tat, gp120), as well as HIV-induced immune dysregulation, contribute to pulmonary vascular disease, particularly through an impact on pulmonary endothelial cells (EC). HIV Nef protein co-localizes with EC in PAH- like plexiform lesions in animal models [20]. Substance abuse also plays a significant role. In animal models, cocaine increases proliferation of pulmonary vascular endothelial cells and morphine contributes to vascular disease and oxidant stress [21;22].

HIV AND LUNG CANCER

Lung cancer is the primary cause of cancer related death among PLWH [8]. Low CD4+ T cell counts and prior pneumonia are associated with lung cancer risk [23;24]. Cigarette smoke, HIV infection and chronic inflammation can increase oxidative stress, leading to oxidative DNA

lesions and DNA double strand breaks. Incorporation of the HIV genome into infected cells is dependent on host cell DNA repair proteins, and HIV-induced alterations in DNA repair proteins have been implicated in augmenting genomic integration and replication in host cells [25]. HIV Tat protein *in vitro* can induce expression of pro-oncogenes (c-myc, c-fos, c-jun) and downregulate the p53 tumor suppressor gene [26]. Although cART can have genotoxic effects, no association has been found between cART and lung cancer risk, and early cART is associated with decreased risk [27].

COMMON MECHANISMS OF LUNG DISEASE IN PLWH

The cause of higher rates of noninfectious lung complications in PLWH is multifactorial. PLWH demonstrate evidence of chronic lung inflammation from a variety of causes, including direct effects of HIV, cART, illicit drug use, immunodeficiency, opportunistic infections, and alterations in the lung microbiome. The potential mechanisms by which these factors contribute both specifically and generally to HIV-PAH, COPD and lung cancer are outlined in Figure 1.

SMOKING AND HIV

The prevalence of smoking in PLWH ranges from similar to twice that of the general population depending on the comparison group. The largest study demonstrated that 42% of PLWH are current smokers, compared to 21% of the general population [28]. In contrast, when the comparison group consists of uninfected subjects with a high smoking prevalence, such as the Veterans' Aging Cohort Study (VACS) or Multicenter AIDS Cohort Study (MACS) cohort which matches patients with similar risk profiles, smoking rates are more similar in the two

groups, although PLWH still have higher rates [29;30]. Compared to non-smokers, PLWH who smoke have higher levels of inflammatory markers, including soluble CD14 and expression of HLA-DR on both CD8+ and CD4+ T cells [31;32]. Cigarette smoking has been linked to increased rates of mortality due to cardiovascular disease, COPD, pneumonia, and lung cancer [29]. If HIV is treated, modeling studies suggest that PLWH who smoke lose over six years of life expectancy, more than that lost to HIV infection [33].

PLWH are less likely to quit than the general population (32% vs 52%) [28]. Multiple factors are associated with continued smoking in PLWH, including higher rates of other substance abuse, psychiatric disorders, low socioeconomic status and poor access to care [34]. Perhaps because of multiple competing interests and limited time to address medical issues, counseling on smoking cessation happens less frequently for PLWH than the general population [35].

Despite clear evidence that cigarette smoking contributes to significant disease in PLWH, most studies of smoking related illness are poorly controlled for the impact of cumulative exposure to cigarette smoke, and control only for smoking status, i.e. current, past or nonsmoking status. A recent study clearly demonstrated that both cumulative pack-year smoking history and time since smoking cessation are more strongly associated with lung and heart disease than smoking status [36], suggesting that a true understanding of the role of cigarette smoke as a pathogenic factor in HIV-associated lung disease will require better collection of data on total tobacco exposure.

HIV INFECTION

HIV pathology in the lung is driven by infection of CD4+ T cells and alveolar macrophages, which play an important role in the development of pulmonary disease [37;38]. Animal models demonstrate that the lungs and intestines harbor the highest levels of simian immunodeficiency virus (SIV) among non-lymphoid tissues [39], and early in infection, CD4+ T cells are rapidly depleted from mucosal sites [37;40]. Recent data suggests that lung epithelium can also be directly infected by HIV, especially CXCR4-tropic strains associated with advanced disease. HIV infection results in integration of the viral genetic material into the cellular genome. This integration may change gene expression and immune response [41]. Expression of viral proteins, such as Tat, increases inflammation and oxidative stress in animal models [42]. HIV infection alters the function of airway epithelial cells by impairing cell-cell adhesion and increasing the expression of inflammatory mediators [43]. Thus HIV infection contributes to lung disease by both direct effects of infection and through modulation of systemic inflammation and immunodeficiency.

COMBINATION ANTIRETROVIRAL THERAPY (cART)

Treatment of HIV-1 infection with cART has generally been associated with improved outcomes [29], especially in reduction of infectious pulmonary complications. Its influence on non-infectious pulmonary complications has been more controversial. Protease inhibitor use has been linked to an increased incidence of malignancy, although not lung cancer specifically [27]. Older antiretrovirals may have had genotoxic effects which contribute to this increased risk. Although one study demonstrated that cART increased the risk of COPD [44], the pulmonary substudy embedded in the large Strategic Timing of Antiretroviral Therapy (START) trial demonstrated that the timing of ART had no effect on COPD progression [45], and poor HIV control in a predominant smoking population is associated with the development of HIV-associated COPD and accelerated annual lung decline

[46]. Antiretroviral use improves hemodynamics and survival in HIV-PAH, although not as dramatically as PAH therapy [18;47].

INFLAMMATION IN THE LUNG

From early in the epidemic, it was noted that HIV infection is associated with a CD8+ T cell alveolitis, that occurs in both asymptomatic patients and those with respiratory symptoms and HIV disease progression [48]. Many of these cytotoxic T cells are directed against HIV-infected cells or other opportunistic pathogens (i.e. cytomegalovirus, *Pneumocystis*) [49;50]. Lung CD8+ T cells appear to be dysfunctional, expressing high levels of the exhaustion marker, PD-1, in the absence of antiviral therapy [49]. Lymphocytes expressing exhaustion markers such as PD-1 and CD57 are thought to be terminally differentiated senescent cells. However, CD8+ T cells maintain the capacity to secrete a pro-inflammatory effector cytokines in response to HIV antigens under conditions of poor viral control as well as following viral suppression [12;49;51]. Thus, the presence of terminally differentiated effector lymphocytes in the lung contributes to local inflammation in response to HIV itself, as well as other pathogens. HIV infection can cause lung inflammation in other ways as well. For example, alveolar macrophages (AM) are infected by HIV, even in healthy, nonsmoking PLWH, and infected cells have impaired phagocytic function as well as abnormal oxidative burst and cytokine secretion [52;53]. Untreated PLWH who smoke showed significant lung CD4+ T cell dysfunction and depletion, along with high susceptibility to apoptosis, which improved following cART [51].

Thus, HIV-associated lung disease in PLWH likely is driven by multiple inflammatory mechanisms: 1) HIV replication in lung CD4+ T cells and AM, 2) CD8+ T cell alveolitis and an

imbalance of the physiologic lung CD4:CD8 ratio, 3) progressive CD4+ T cell depletion/dysfunction, 4) dysregulation of alveolar macrophages, and 5) impaired immune function to other pathogens, which predisposes to infection and parenchymal damage. Together with tobacco exposure, this combination of cellular activation and immune dysfunction contributes to the pathogenesis of lung disease.

HIV ASSOCIATED PULMONARY INFECTIONS AND THE MICROBIOME

Recent studies have revolutionized our understanding of the role the microbiome plays in health and disease [54]. Although the microbial community is smaller in the lung than in the gut, it is clear that it is diverse and modulated by disease [55;56]. Pulmonary flora is modulated by inhaled corticosteroids or bronchodilator use [56], while smoking has minimal effects on the lung microbiome [56;57]. In general, most inflammatory disease states, such as COPD, are associated with a decline in microbiome diversity, and increased abundance of inflammatory Proteobacteria species, such as *Pseudomonas, Moraxella*, and *Haemophilus influenzae* [55;56].

Changes in the bacterial microbiome of the lung have been described in HIV infection, and may account for increased pathology at both these sites particularly with advanced immunodeficiency. The first study of the lung microbiome in HIV infection demonstrated an increased prevalence of *Tropheryma whipplei* in untreated subjects, although a broader comprehensive analysis of upper and lower bacterial microbiomes using 16S rRNA methods did not demonstrate a significant difference in bacterial communities between healthy PLWH and uninfected individuals [58;59]. In contrast, PLWH with low CD4+ T cell counts have fewer numbers of species (decreased alpha diversity), although more unique species are present (increased

beta diversity) [59;60]. The HIV lung microbiome contains increased amounts of *Veillonella* and *Prevotella*, bacteria previously shown to be associated with inflammation [61]. An increase in detection of *Pneumocystis jiroveci* is associated with both HIV infection and COPD [62].

HIV pathogenesis is characterized by gastrointestinal CD4+ T cell depletion and a compromised mucosal barrier, leading to microbial translocation, endotoxemia and systemic immune activation [40]. Shifts in the gut microbiome have also been seen in PLWH [63;11], although the extent to which this is due to HIV infection versus other factors, such as sexual preference, is still unclear[64]. In addition to systemic immune activation, which appears to be related to gastrointestinal pathology, changes in the gut microbiome may have a particular influence on pulmonary disease. Several epidemiologic studies, as well as studies in murine models, have demonstrated a correlation between susceptibility to pulmonary infections, allergic airway disease and an altered fecal microbiome [65]. There is an epidemiologic correlation between COPD and inflammatory bowel disease [66], which may be smoking related, but may also be due to shifts in the microbiome, inflammation or modulation of matrix metalloproteinases [67].

OXIDATIVE STRESS

The lung is at particular risk of damage due to excessive oxidative stress, because it is directly exposed to oxygen, inhaled pollutants, and microbes that produce pro-oxidant reactive oxygen and nitrogen species. Higher levels of markers of oxidative stress and lower levels of antioxidant glutathione are found in smokers, as well as patients with COPD and/or HIV [68]. Oxidative stress is worsened during acute exacerbations of COPD [69]. HIV infection is associated with high rates of oxidative stress [68], which may be due to the direct effects of the viral

proteins, gp120 and tat, on lung epithelium [42]. Alcohol and tobacco use by HIV-infected patients contribute to oxidative stress, potentially through the involvement of antioxidant pathways and the cytochrome P450 system [70].

MATRIX METALLOPROTEINASES (MMP)

MMPs are a large family of zinc-dependent endopeptidases that can cleave the majority of structural components of the extracellular matrix, including elastin. Studies have identified increased proteolytic activity in PLWH who smoke and have emphysema [13], and examination of their BAL confirmed elevated expression of MMP mRNA in alveolar macrophages, and MMP protein and activity in supernatant. Recent studies have demonstrated that high levels of inflammatory cytokines, such as IL-23, are seen in the BAL of these patients. Infection of AM results in expression of IL-23, which can lead to upregulation of MMP 9 in AM in an AM/lymphocyte co-culture model [14]. Studies of human macrophages have consistently located MMP-12 and MMP-9 in emphysematous lung [71]. Murine models have demonstrated that macrophage overexpression of MMP-9 or MMP-1 can spontaneously induce emphysema, while MMP-12 knockdown is protective from cigarette smoke-induced emphysema [72]. MMP expression is additionally upregulated and has been demonstrated to play an important role in the development of PAH in animal models [73]. It is notable that MMP-12 is quite responsive to smoking cessation and if this protease has an exaggerated role in HIV-mediated lung disease, smoking cessation may result in an even more significant impact in this cohort [74].

ADDRESSING MOLECULAR MECHANISMS OF LUNG DISEASE IN HIV

We currently are at a critical point in investigating lung disease in PLWH. The continued development of less toxic cART has resulted in more persistent and robust recovery of immune function. Nonetheless, a recent study highlighted both a significant under-prescription of cART and ineffective viral suppression in PLWH [75]. The institution of more generalized screening of individuals at risk for HIV has resulted in fewer opportunistic infections and specialized care earlier in disease. Although issues like smoking and IVDU are difficult to treat, the epidemiology from several large cohorts has made the potential size of our future problem quite clear. At this time a major knowledge gap remains whether lung disease is occurring at a very rapid rate prior to treatment or if there is continued accelerated decline with disease even on cART. In addition, cohorts comprised of subjects over age 40 are needed to longitudinally assess lung T cell subsets, antigen-specific immunity including HIV-specific responses, macrophage activation and *Pneumocystis* colonization to determine which of these predict progression of lung disease. Further, there is a need to study patients who have not had significant immune suppression and opportunistic infections to evaluate whether they are at high risk of lung disease in the absence of advanced immunodeficiency.

Another unmet need is the execution of well-designed mechanistic studies on the pathogenesis of lung disease. To understand the mechanisms of HIV-associated inflammation, we must define the relative importance of a variety of potential causes of inflammation: HIV itself; tobacco, alcohol and illicit drug use; opportunistic infections; shifts in the microbiome to organisms capable of causing chronic inflammation (bacteria such as *Prevotella*, persistent latent virus infection, persistent fungi such as *Pneumocystis*), current antiretroviral drugs, or other as yet unidentified mechanisms. Understanding these factors may be critical to our

understanding of other inflammatory disorders in PLWH, and may allow us to develop therapeutic interventions to alter the progression of disease.

The INvestigating HIV-Associated Lung Disease (INHALD) consortium (http://statepiaps.jhsph.edu/lamdacc/inhald/index.html) is a collaborative effort designed to evaluate and understand the mechanisms of lung disease in PLWH. The INHALD cohort will provide invaluable data to assist in our understanding of lung disease in patients during the modern era of cART, and will generate information about specific downstream mechanisms. . Sites are conducting assessments including pulmonary function testing, imaging, and echocardiography. Biologic samples, including blood, peripheral blood mononuclear cells (PBMC) and BAL are being collected and banked. Sites are collaborating on projects to evaluate the microbiome (with a particular focus on virome and shotgun metagenomics), inflammation, and oxidative stress, and welcome outside collaborations. As the HIV-infected population ages, lung disease is likely to become an increasingly common comorbidity given high rates of smoking in this population. Appropriate management will require an understanding of modifiable mechanisms of disease to define best practices.

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